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A Novel Twist in Membrane dePHormation

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Bin-Amphiphysin-Rvs (BAR) domain-containing proteins form oligomeric assemblies that aid membrane remodeling. In this issue of *Developmental Cell*, Pang et al. (2014) show that the BAR domain of ACAP1, although architecturally similar to other BAR domains, cooperates with its neighboring pleckstrin homology domain to deform membranes and facilitate endosomal recycling.

Vesicular transport and other cellular processes require extensive lipid membrane remodeling by protein scaffolds, such as the Bin-Amphiphysin-Rvs (BAR) domain-containing proteins. BAR domains form dimeric α-helical coiled coils that bind to acidic membrane phospholipids to actively stabilize or induce membrane curvature (Daumke et al., 2014; Frost et al., 2009; Peter et al., 2004). Self-assembly of BAR domain dimers into larger-scale scaffolds through low-affinity tip-to-tip and/or lateral interactions (Frost et al., 2009; Mim et al., 2012) is well known to influence the shape of the underlying membrane. The membrane remodeling function of BAR domains can be modulated by additional elements, including hydrophobic wedge loops or amphipathic helices nested into or flanking the BAR domain that insert into the cytosolic leaflet of the lipid bilayer (Daumke et al., 2014; Frost et al., 2009; Peter et al., 2004). Now in *Developmental Cell*, Sun and colleagues (Pang et al., 2014) add a surprising level of complexity to our understanding of BAR domain-dependent membrane remodeling. The authors show that the BAR domain of ACAP1, a GTPase-activating protein involved in endosomal recycling, does not directly contact the membrane and instead acts solely as a multimerization domain to enable membrane deformation by the neighboring pleckstrin homology (PH) domain.

The function of BAR domain proteins has been well established in endocytosis. Specific BAR domain proteins interact with endocytic vesicle intermediates, which display different curvatures, to enable membrane fission mediated by the GTPase dynamin (Daumke et al., 2014). Several other pathways also utilize BAR domain protein scaffolds, yet, in many cases, it is unclear how assembly of these proteins facilitates membrane remodeling. As one example, tubular transport carriers facilitate ER-to-Golgi transport and also emanate from perinuclear endosomes (Li et al., 2007) during slow endocytic recycling of subsets of integrins and other proteins. Formation of these tubule carriers depends on the activity of ADP-ribosylation factor 6 (ARF6), which itself is regulated by guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) (Donaldson and Jackson, 2011). The Arf6-GAP ACAP1 (ArfGAP with Coiled-coil, Ankyrin repeat and Pleckstrin homology domain) regulates endosomal recycling of several proteins (Li et al., 2007) and, when overexpressed in cells, is found on the surface of tubular carriers emanating from recycling endosomes. ACAP1 is then incorporated into ARF6-dependent clathrin coats and imposes membrane curvature, a property previously attributed to its BAR and PH domains (Li et al., 2007). Although PH domains promote binding to phosphoinositides, why the membrane tubulating activity of ACAP1 requires the presence of the neighboring PH domain is unclear.

Pang et al. (2014) set out to unravel the molecular mechanisms underlying the generation of membrane curvature by ACAP1. The authors applied X-ray crystallography to determine the 3D structure of ACAP1$^{BAR\text{-}PH}$. The ACAP1^{BAR} domain exhibits all of the typical features of BAR domains, including the propensity to form banana-shaped dimers with basic patches exposed at the concave side, as well as the presence of an amphipathic helix at the N terminus that might allow for membrane deformation. Surprisingly, however, ACAP1^{BAR} proved unable to bind or deform liposome membranes in vitro. Mutation of basic residues at the concave surface of the BAR domain within ACAP1$^{BAR\text{-}PH}$ hardly affected membrane association, whereas mutating basic residues within the PH domain completely abrogated membrane binding and deformation. Thus, the adjacent PH domain, rather than the BAR domain itself, tethers ACAP1$^{BAR\text{-}PH}$ to membranes.

The PH domains are positioned at the ends of the BAR domain dimer, to which they are connected through flexible linkers. Upon closer inspection, the authors noted an additional important feature of the PH domain: a hydrophobic loop in the vicinity of the basic patch. PH domains containing such a wedge-like feature have previously been implicated in membrane deformation (Lenoir et al., 2010), and mutation of residues in this loop abolished membrane deformation induced by ACAP1$^{BAR\text{-}PH}$. An ACAP1 mutant, in which one hydrophobic residue was exchanged (F280E), was consistently unable to facilitate recycling vesicle formation from endosomes in a reconstituted system in vitro.

This raises the question of what function the BAR domain of ACAP1 plays in membrane remodeling. To answer this question, Pang et al. performed cryoelectron microscopy (cryo-EM) analysis of liposomes. Upon incubation with the ACAP1$^{BAR\text{-}PH}$ domain, liposomes form 40–50 nm diameter membrane tubules coated with helical arrays of ACAP1$^{BAR\text{-}PH}$. Low-resolution cryo-EM maps, in combination with information from their X-ray crystallography, allowed the authors to build a structural model of ACAP1$^{BAR\text{-}PH}$ coats. These analyses revealed that ACAP1$^{BAR\text{-}PH}$ assembles into tetramers with an elongated banana shape, with two PH domains associated with the membrane surface and two PH domains protruding away from the membrane.

Only one PH domain of a given tetramer inserts its hydrophobic loop into the membrane. ACAP1^{BAR-PH} tetramers are packed such that PH domains close to the membrane are covered by the BAR domain assembly of a neighboring tetramer. Strikingly, no significant interaction of the BAR domain with the underlying membrane was observed, in agreement with the proposal that the BAR domain itself does not serve as the major tether that anchors ACAP1 at membranes.

To further confirm their structural model, the authors generated an artificial fusion version of ACAP1^{BAR-PH} containing tandem repeats of the BAR-PH monomer. In this fusion protein, the overall orientation of the monomers is preserved and the central BAR domains are flanked on either side by PH domains, as in the native protein. These fusion dimers retained their ability to induce membrane tubulation, even if one of the two PH domains was rendered inactive by mutating F280. Together with molecular simulation data, these results demonstrate that ACAP1 induces membrane deformation through a cooperative mechanism in which individual ACAP1^{BAR-PH} dimers behave asymmetrically. When approaching the membrane, the BAR domains in the dimer adopt a conformation that accurately positions only one of the PH domains toward the lipid bilayer. This PH domain binds to phosphoinositides and inserts its hydrophobic wedge loop into the membrane to ultimately facilitate remodeling.

In some respects, ACAP1^{BAR-PH} architecturally resembles the complex formed by the GTPase dynamin, which functions in endocytic vesicle fission. In the dynamin complex, an antiparallel four-helix bundle called the stalk is arranged in a crisscross fashion and serves as the major platform for dynamin oligomer assembly (Daumke et al., 2014; Faelber et al., 2011). Upon oligomeric assembly, the PH domains adjoining the stalk are dislodged and reorient toward the membrane to facilitate membrane deformation and possibly vesicle fission. While ACAP1 does not contain a GTPase domain like dynamin, it serves as a GAP for the small GTPase ARF6, which likely cooperates with ACAP1 in mediating the formation of recycling vesicles from endosomes. Unravelling the contribution of ARF6 to this process and the precise mechanism of membrane fission clearly remains a major challenge for future studies.

The tandem arrangement of BAR and PH domains is not unique to ACAP1. A number of other scaffolding proteins involved in membrane trafficking and endocytosis, including other ArfGAPs, APPLs, and sorting nexins, contain similar arrangements of BAR and PH, or the related PX, domains (Frost et al., 2009). Some of these factors have also been shown to tubulate membranes (Knævelsrud et al., 2013). Thus, the mechanism of membrane deformation elucidated by Pang et al. may be relevant for other membrane-trafficking pathways, and future work will provide additional insights into the complexities of membrane remodeling.

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